CAR19 T Cells Redirected to Novel Antigens Mediate Robust Cytotoxicity Against Diverse Malignancies

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1 - Introduction
Adoptive cellular therapy can cure advanced B cell leukemias and lymphomas. This progress has been made using CARs that recognize B cell antigens, particularly CD19, referred to here as CAR19 T cells. One component of the unique success of CAR19 adoptive cellular therapy is the provision of antigen and productive co-stimulation by normal CD19+ B cells in protected niches, and exiting the bone marrow post-lymphodepletion, allowing the CAR19 T cell population to persist for many months or even longer. However, an emerging issue in the treatment of advanced B cell malignancies with CAR19 cellular therapeutics is loss of CD19 antigen on the target tumor cells, and subsequent patient relapse. Further, and in contrast with CD19+ B cell malignancies, progress against other cancers has been limited. Here we present a novel strategy to leverage the potency and persistence of CAR19 T cells by redirecting their cytotoxic activity to novel tumor antigens. The technology, termed IMPACT™ (Integrated Modular Proteins for Adoptive Cell Therapy) can be applied to diverse antigens and tumor types. We present therapeutic modalities that address the issue of antigen loss in B cell leukemias & lymphomas, and that enable the targeting of diverse antigens in CLL, AML and multiple myeloma.

2 - Technology Overview
IMPACT fusion proteins (FP) are created by cloning the extracellular domain (ECD) of a CAR T cell target protein (e.g. CD19) into an scFv that recognizes a tumor associated antigen (TAA). The system is modular: diverse ECD-scFv fusion proteins have been designed and expressed. The FP create a bridge between the CAR19 T cell and the target antigen+ cell (Figure 1).

Figure 1. Bridging CAR19 T cells to antigen+ tumor cells

3 – Delivery of FP to the target tumor
We have developed three distinct methods to deliver FP to the tumor microenvironment.

4 – FP Activity
Here we use soluble FP to investigate the biologic properties inherent in the system, a prerequisite to developing the CAR19 integrated FP modules as therapeutics. FP affinities for targets (anti-CD19 / CD19-ECD; anti-TAA / TAA) are measured in ELISA and flow cytometry assays, and the potency of the FP is assessed in cytotoxicity assays (Figures 2, 3).

Figure 2. Affinity of the CD19-anti-CD20 FP for 293-CD20 cells, as detected with anti-CD19-fluorescent antibody (FMC63-PE)

5 – CAR19 with the IMPACT™ FP encoded as an integrated gene
The development candidates are being constructed as integrated genes (i-gene FP) using lentiviral vectors and packaging systems. A prototype schematic is shown here:

Table 2. Programs in development.

6 – Serial cytotoxicity

7 – Conclusions and path forward
IMPACT™ fusion proteins mediate redirected tumor cell killing and can be successfully secreted from CAR T cells well in excess of the concentration required for cytotoxicity. The first in vivo studies using CAR19 cells carrying specific i-gene FP cassettes are in life.

The degree of cytotoxicity observed is similar regardless of the “direction” of serial killing in Nalm > Skov or Skov > Nalm. Preliminary data indicate that engagement of Nalm (a CD19+ B cell) provides important co-stimulatory signals and improves the phenotype of the CAR19 T cell, with properties consistent with robust expansion and persistence (preliminary data not shown).

Table 1. Potency analysis of diverse FP therapeutics.

Table 4. Programs in development.

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